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Comparison of Hollow-Fiber Ultrafilters with Pleated Capsule Filters for Surface and Tap Water Samples Using U.S. EPA Method 1623

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Abstract

The EPA method 1623 is designed specifically for the detection of *Cryptosporidium* and *Giardia*, but the method has some issues with low and variable recoveries. Ultrafiltration has been used effectively for microorganism recovery from water samples but is not approved by the EPA. To determine the efficacy of using ultrafiltration, 10-L tap water and surface water samples were seeded with *Cryptosporidium* and *Giardia* and concentrated with either a pleated capsule filter or a hollow-fiber ultrafilter. For *Cryptosporidium*, oocyst recovery in tap water was significantly higher for ultrafiltration (68%) versus the capsule filter (37%); ultrafiltration recovered 65% of oocysts in surface water versus 61% for the capsule filter. However, *Giardia* cyst recovery was mixed. In tap water, the capsule filter produced a significantly better recovery (85%) of *Giardia* compared with ultrafiltration (63%), but the surface water ultrafiltration recovery (81%) was significantly better than the capsule filter recovery (40%). Overall, ultrafiltration recoveries were equal to or better for *Cryptosporidium*, but recoveries of *Giardia* were varied depending on the filter used and the type of water analyzed.

Author keywords

Drinking water; Surface water; Microbes; Laboratory tests; Methodology

Introduction

Cryptosporidium and *Giardia* are protozoa that can be present in surface water and can remain after conventional drinking water treatment processes, including disinfection with chlorine. Outbreaks of *Cryptosporidium* associated with drinking water have been documented in the United States since the early 1980s (Solo-Gabriele and Neumeister 1996). In 1993, an outbreak of *Cryptosporidium* in the municipal drinking water supply made more than 400,000 people ill and killed approximately 100 individuals in Milwaukee (MacKenzie et al. 1994). For healthy individuals, this illness can produce gastrointestinal symptoms, because the immune system works to fight off the infection. However, cryptosporidiosis can be fatal in immunocompromised individuals whose immune systems cannot fight the infection. Therefore, it is imperative that reliable methods be available for analysis of drinking water protect the public from waterborne *Cryptosporidium*.

When the Milwaukee outbreak occurred in 1993, *Cryptosporidium* testing requirements did not exist for public water suppliers. However, with the promulgation of the Long Term 2 Enhanced Surface Water Treatment Rule (LT2 rule), surface water monitoring for *Cryptosporidium* became a requirement for water suppliers with a population of 10,000 or greater (U.S. EPA 2006). Public water suppliers that were required to sample under the LT2 rule had to perform monthly monitoring for 24 months.

Neither *Cryptosporidium* nor *Giardia* can be detected with fecal indicator-organism tests that are common in the water industry, nor has any correlation been shown between the detection of fecal indicator organisms and either *Cryptosporidium* or *Giardia* (Sobsey 1989). To detect these organisms, a specific method of analysis must be used. Currently, the approved method for the detection and analysis of these two microorganisms is EPA method 1623 (U.S. EPA 1999). There can be problems with recovery and variability when method 1623 is used (DiGiorgio et al. 2002; Hu et al. 2004). Thus, researchers have tried different modifications to improve the performance of method 1623. One method alteration that has been tested involves the type of filter used. The most commonly used filter in method 1623 is a pleated capsule filter, the Envirochek HV (Pall Corporation, Ann Arbor, MI), but some researchers have tested the efficacy of hollow-fiber ultrafilters (Hill et al. 2005, 2009; Morales-Morales et al. 2003; Simmons et al. 2001).

One study that compared the standard Envirochek filter with a hollow-fiber ultrafilter (Fresenius Hemoflow F80A, Fresenius Medical Care, Lexington, MA) for processing both reagent and surface water samples with EPA method 1622 (*Cryptosporidium* only) reported no significant difference between the filters when processing reagent water, but the ultrafilter performed significantly better when surface water was analyzed (Simmons et al. 2001). A second study using the same type of hollow-fiber ultrafilter reported recoveries of greater than 80% for *C. parvum* from seeded tap water samples (Hill et al. 2005). A more recent study that compared ultrafiltration (UF) with method 1623 found that UF produced significantly better recoveries of *Cryptosporidium*, but not *Giardia*, in tap water samples (Hill et al. 2009).

The objective of this study was to determine the recovery of *Cryptosporidium* and *Giardia* from both surface water and tap water using the Envirochek HV (Pall Corporation, Ann Arbor, MI) and Fresenius Optiflux 200NR filters (Fresenius Medical Care, Lexington, MA). Fresenius Optiflux 200NR filters are high-flux, hollow-fiber, polysulfone dialysis filters with a surface area of 2.0 m², a fiber inner diameter of 200 μ m, and a molecular weight cutoff of approximately 30 kDa; these filters were operated in the cross-flow mode for this study. Hollow-fiber ultrafilters have not gone through a Tier 2 validation study for approval by the EPA for use with method 1623. However, UF can be validated under the EPA performance-based measurement system through completion of a Tier 1 validation study as long as acceptance criteria are met.

Methods

Ten-liter tap water ($n = 5$ per filter type) and source water ($n = 5$ per filter type) samples were obtained from the Franklin Water Treatment Plant (Charlotte, NC), whose surface water comes from Mountain Island Lake. Turbidity for the tap water is generally in the range of 0.1–0.3 nephelometric turbidity units (NTUs), whereas the source water generally has turbidity values of less than 5 NTUs. Total organic carbon (TOC) averages approximately 1 mg/L in the tap water and is less than 2 mg/L in the source water. Flow-cytometry sorted oocyst/cyst suspensions obtained from the Wisconsin State Laboratory of Hygiene (Madison, WI) were seeded into the water samples. Different sets of spiking suspensions were used during the sample analyses, but each 15-mL suspension contained a specified number of cysts/oocysts in the range of 149–172, according to the associated specification sheets that were supplied with the suspensions.

Five tap water samples were processed with the pleated capsule filter, and five tap water samples were processed with the hollow-fiber ultrafilter. Similarly, five surface water samples were processed with each filter type. In addition, unseeded control samples of each water type were also processed, and neither *Cryptosporidium* nor *Giardia* was detected in any of the control samples.

Filtration of samples through the pleated capsule filters was performed with a diaphragm pump (Shurflo, Cypress, CA), and UF was performed with a peristaltic pump (Cole Parmer Instrument Company, Vernon Hills, IL). After filtration, samples were processed using method 1623 techniques, with the exception of the elution procedure. The pleated capsule filters were eluted as specified in method 1623, but the ultrafilters were backwashed according to the procedure used by Hill et al. (2005), with a solution that contained 0.2% Tween 80, 0.01% sodium polyphosphate, and 0.01% Antifoam A. Following concentration by centrifugation at $1,500 \times g$ and aspiration of the supernatant, each sample was further processed using immunomagnetic separation (Dynabeads GC-Combo, Invitrogen Dynal, Oslo, Norway), and slides were stained (EasyStain, BTF, Sydney, Australia) according to the procedures in method 1623.

The two filtration methods are similar in the amount of time required for completion. The pleated capsule filtration required approximately 10 min, and the time required for the UF procedure was approximately 15–20 min. Because elution/backwash procedures were also

different for the two types of filters, the time required for this step also varied slightly between the filter types. The elution procedure performed on the pleated capsule filter can be completed in 20–25 min, and the backwash procedure performed on the hollow-fiber filter can be completed in 5–10 min. Overall, each method can be completed in approximately 5–6h.

Recovery efficiency for each sample was calculated by dividing the number of recovered organisms by the number of organisms seeded into the 10-L sample. The resulting fraction was then multiplied by 100 to obtain a percent recovery. Statistical comparisons were made using one-way ANOVA with statistical significance set at 0.05 (Minitab 15, State College, PA).

Results and Discussion

For the tap water samples ($n = 5$ per filter type), as shown in Fig. 1, the mean recovery of *C. parvum* for the pleated capsule filters was 37% (SD = 17), whereas the use of the ultrafilters achieved a significantly higher ($p = 0.007$) mean recovery of 68% (SD = 10) for *C. parvum*. For *Giardia*, the pleated capsule filters produced a mean recovery of 85% (SD = 6), and UF achieved a mean recovery of 63% (SD = 8; $p = 0.001$).

Fig. 2 shows the mean recoveries for each filter type by organism in surface water samples. For the surface water samples ($n = 5$ per filter type), the mean recovery of *C. parvum* was 61% (SD = 14) when using pleated capsule filters and 65% (SD = 7) when using UF. No statistically significant difference was found between the recoveries ($p = 0.63$). For *G. intestinalis*, recoveries in surface water for the pleated capsule filters averaged 40% (SD = 12). However, UF achieved a significantly higher mean recovery of 81% (SD = 5) for *Giardia* in surface water ($p = 0.00009$).

When compared with previous research, this study has produced similar and dissimilar results. As found in the current study, Hill et al. (2009) reported that UF produced significantly better recoveries of *Cryptosporidium* but not *Giardia* in tap water samples. Conversely, although the current study did not find a difference in recoveries of *Cryptosporidium* in surface water, Simmons et al. (2001) reported significantly better recoveries of *Cryptosporidium* with UF. However, these two studies used different models of ultrafilters and pleated capsule filters.

Conclusions

The results from this study demonstrate that UF can provide similar or better recoveries of *Cryptosporidium* and *Giardia* than recoveries from pleated capsule filters when applied to surface water. When applied to tap water samples, UF recoveries were significantly better than Envirochek HV filters for *Cryptosporidium*, but *Giardia* recoveries were better with the Envirochek HV (although overall method recoveries with UF were still greater than 60%). Solely on the basis of the results of this study with one single surface water, UF may be a viable option to improve *Cryptosporidium* and *Giardia* recoveries from both surface and tap water samples using EPA method 1623, but more samples of these and other types and sources of water need to be examined.

Acknowledgments

The use of trade names and names of commercial sources is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention or the U.S. Department of Health and Human Services. The findings and conclusions in this presentation are those of the authors and do not necessarily represent those of the Centers for Disease Control and Prevention.

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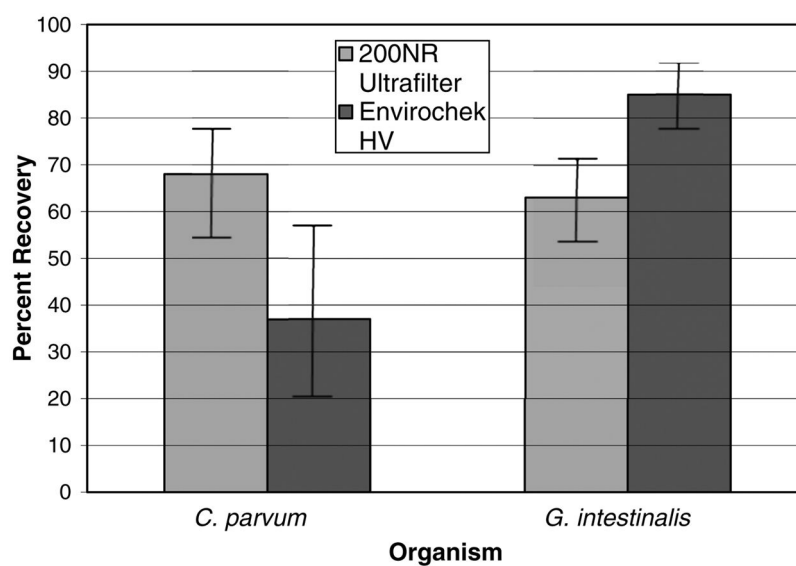


Fig. 1.
Mean recovery of *Cryptosporidium parvum* and *Giardia intestinalis* in tap water samples for each filter type (error bars represent standard deviation)

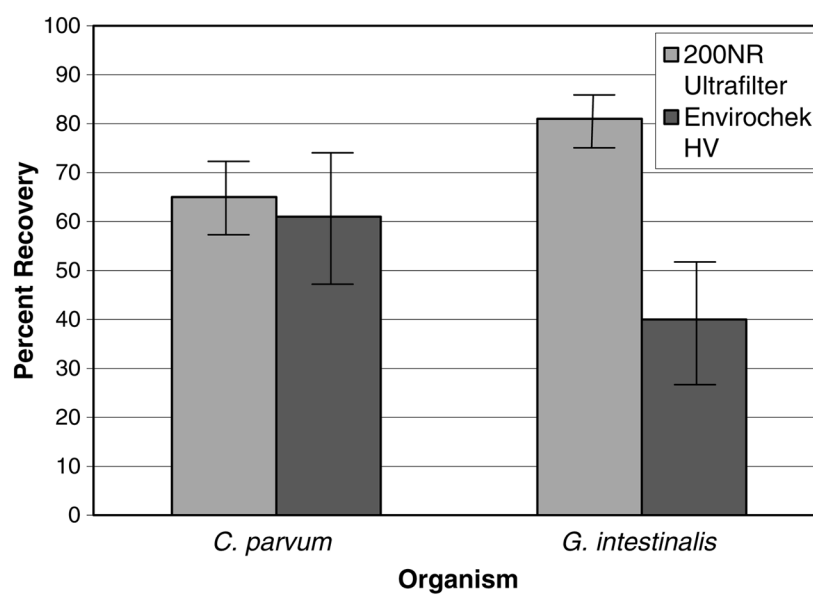


Fig. 2.
Mean recovery of *Cryptosporidium parvum* and *Giardia intestinalis* in surface water samples for each filter type (error bars represent standard deviation)